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**Glucose control upon waking is unaffected by hourly sleep fragmentation during the night,
but is impaired by morning caffeinated coffee.**

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Sleep fragmentation and caffeinated coffee

Keywords: Sleep Fragmentation, Coffee, Caffeine, Glucose, Insulin, CYP1A2, Polymorphism

50 **Abstract**

51 Morning coffee is a common remedy following disrupted sleep yet each factor can independently
52 impair glucose tolerance and insulin sensitivity in healthy adults. Remarkably, the combined effects
53 of sleep fragmentation and coffee on glucose control upon waking *per se* have never been
54 investigated.

55 In a randomised cross-over design, 29 adults (Mean \pm SD; age: 21 ± 1 years, BMI: 24.4 ± 3.3 kg·m⁻²)
56 underwent three oral glucose tolerance tests (OGTT). One following a habitual night of sleep
57 (*Control*; in bed, lights-off trying to sleep ~2300-0700 h) the others following a night of sleep
58 fragmentation (as *Control* but waking hourly for 5 min), once with and once without morning coffee
59 ~1 h after waking (~300 mg caffeine as black coffee 30 min prior to OGTT).

60 Peak plasma glucose and insulin concentrations were unaffected by sleep quality but were higher
61 following coffee consumption (Mean [normalised confidence interval] for Control, Fragmented, and
62 Fragmented+Coffee, respectively; Glucose: 8.20 [7.93-8.47] mmol·L⁻¹ *versus* 8.23 [7.96-8.50]
63 mmol·L⁻¹ *versus* 8.96 [8.70-9.22] mmol·L⁻¹; Insulin: 265 [247-283] pmol·L⁻¹; and 235 [218-253]
64 pmol·L⁻¹; and 310 [284-337] pmol·L⁻¹). Likewise, iAUC for plasma glucose was higher in the
65 Fragmented+Coffee trial compared to Fragmented.

66 Whilst sleep fragmentation did not alter glycaemic or insulinaemic responses to morning glucose
67 ingestion, if a strong caffeinated coffee is consumed then a reduction in glucose tolerance can be
68 expected.

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Introduction

Sleep curtailment is a risk factor for obesity-associated metabolic diseases, possibly due to the important role of sleep in maintaining glucose homeostasis [1]. For example, impaired glucose clearance and whole-body insulin sensitivity can occur following a single night of either sleep deprivation (e.g. limiting the habitual duration), broken sleep (i.e. 2 periods of sleep separated by an extended waking interval - e.g. 2300-0100 h & 0500-0730 h), or nonspecific sleep-fragmentation (e.g. random arousal stimuli throughout the night) [2-5]. Studies to date have employed differing protocols to curtail sleep however, to our knowledge, only one previous study has examined the influence of hourly sleep-fragmentation on postprandial metabolism [6]. In this study, postprandial insulin was lower following breakfast in the fragmentation condition relative to a normal night of sleep. However, whilst the use of metabolic units used in this study allowed for a highly controlled situation in which subjects are in a stable environment, it is important to consider the first night effect of an unfamiliar environment on sleep quality [7], and therefore to see whether these findings translate into more ecologically valid contexts (i.e. participants sleep in their own beds).

From an ecological standpoint, coffee is a commonly used means of combatting feelings of lethargy and fatigue [8], therefore acting as a ‘therapeutic tool’ following disrupted sleep. However, whilst moderate habitual caffeinated coffee consumption is associated with reduced risk of cardiovascular mortality and cancer incidences [9], studies by Moisey *et al* Moisey, Robinson [10] (~62 mg caffeine per 100 mL coffee) and Robertson *et al* Robertson, Clifford [11] (100-400 mg caffeine) demonstrate the potential for a single serving of caffeinated coffee to acutely impair postprandial glucose metabolism in both normal-weight and overweight individuals. This raises the possibility that that coffee consumption could potentiate any negative effects of sleep disruption on glucose metabolism. Furthermore, this effect of caffeine upon postprandial glycaemia seems to be modulated by a single nucleotide polymorphism in the CYP1A2 gene, which codes for an enzyme responsible for caffeine metabolism in the liver [12]. Caffeine is primarily metabolised (>95%) through CYP1A2 activity [13]. However, this modulating role has yet to be investigated under a variety of scenarios [14, 15].

It is remarkable therefore that no study to date has investigated their combined effects upon waking, although one study has investigated the combined influence of caffeinated coffee (65 mg caffeine) *prior to* sleep deprivation on next-day glucose control. Higher fasting serum insulin and increased levels of glucose and insulin were observed after an oral glucose tolerance test (OGTT) following sleep deprivation (4 h in bed) with consumption of caffeinated coffee relative to decaffeinated coffee [16]. However, coffee was consumed prior to sleep, which is not an ecologically valid model of when coffee is usually consumed, especially as a remedy following disrupted sleep. In addition, caffeine is

127 a fast-acting pharmacological agent, with metabolic effects that occur rapidly and may subside within
128 hours (especially amongst habitual caffeine consumers; [17]), so there is also a clear physiological
129 rationale to examine the acute effects of caffeine intake upon waking immediately prior to the first
130 intake of nutrients following the overnight fast.

131
132 The aim of this study, therefore, was to determine the effects of one night of sleep fragmentation with
133 and without morning caffeinated coffee on glycaemic control relative to an undisturbed night of sleep
134 in healthy young adults. An exploratory aim of the study was to examine whether individual responses
135 were mediated by the CYP1A2 genotype of participants. It was hypothesised that sleep fragmentation
136 *per se* would impair insulin sensitivity and that morning coffee would exacerbate this response, with
137 the latter effect modulated by the relevant polymorphism of CYP1A2.

138

139 **Methods and Materials**

140 ***Participants***

141 Twenty-nine healthy men and women (Age: 21 ± 1 years, BMI: $24.4 \pm 3.3 \text{ kg}\cdot\text{m}^{-2}$) participated in the
142 study. Exclusion criteria included, body mass index outside of the range of $18.5\text{-}29.9 \text{ kg}\cdot\text{m}^{-2}$, any
143 diagnosed metabolic disease (e.g. type 1 or type 2 diabetes), reported use of substances which may
144 pose undue personal risk to the participants or introduce bias into the experiment, and non-standard
145 sleep-wake cycle (e.g. shift worker). All were informed of any potential risks and discomfort involved
146 in the study prior to providing written and oral informed consent. The study was given a favourable
147 ethical opinion by the Research Ethics Approval Committee for Health (REACH) at the University
148 of Bath (SES/HES: 18R1-019). The measurements of *CYP1A2* gene polymorphism were completed
149 as part of a wider screening project for which ethical approval was granted by the National Health
150 Service Research Ethics Committee (18/NW/0573). All procedures were performed in accordance
151 with the Declaration of Helsinki.

152 ***Experimental Design***

153 Participants underwent three trials in a randomised cross-over design, with a washout interval of 7-
154 14 days. For 48 h prior to each trial, participants standardised diet and physical activity and refrained
155 from consuming caffeine and alcohol. Main trials involved an oral glucose tolerance test (OGTT)
156 following a habitual night of sleep (Control; in bed, lights-off trying to sleep 8 consecutive hours,
157 waking <1 hour prior to arrival at the lab) and a night of sleep fragmentation (Fragmented; as control
158 but waking hourly for 5 min – prompted and verified by repeated text messaging), with and without
159 morning coffee (Fragmented+Coffee; 300 mg caffeine as black coffee 30 min prior to OGTT).

160 ***Experimental Protocol***

161 Participants arrived in the laboratory at between 0800 h and 1000 h (within 1-hour of waking) in an
162 overnighted fasted state (~10 h). Height, body mass, waist, hip circumference were assessed before
163 participants completed baseline subjective assessments of sleep quality, mood, and appetite on a 0-
164 100 mm scale. Waist and hip circumferences were measured using a tape measure around the mid-
165 point between bottom rib and top of the iliac crest and at the largest circumference between the waist
166 and thighs respectively [18]. An intravenous cannula was placed into an antecubital vein and a
167 baseline sample of 5 mL venous blood collected (BD Venflon Pro; BD, Eysins, Switzerland).
168 Cannulae were kept patent throughout all trials by flushing with 0.9% NaCl infusion (B. Braun;
169 Sheffield, UK). Participants would then either consume a cup of caffeinated coffee (8.8 g Nescafé
170 Original, Nestlé, SUI with 300 mL water; ~300 mg caffeine, ~163 mg total caffeoylquinic acids) or
171 a matched volume of hot water over a 10-min period, 30-minutes prior to undergoing an OGTT. At
172 the end of this 30-minute period a 5 mL blood sample was obtained before ingesting a 75 g oral load
173 of glucose (113 mL Polycal; Nutricia, UK with 87 mL water). Further blood samples were then taken
174 at 15- and 30-minute intervals for the first and second hour of the protocol respectively, alongside
175 hourly assessments of subjective mood and appetite.

176 ***Sleep Fragmentation***

177 Participants were asked to achieve 8-h time in bed, trying to sleep (~2300-0700 h –modifiable
178 according to individual preference and time of testing the next day). Audible alarms were set on the
179 hour every hour throughout the night. Upon waking, participants would receive a series of 10 text
180 messages from a member of the research team, at a rate of 1 every 30 seconds, which required simple
181 responses prior to being able to fall asleep again (e.g. simple arithmetic). This would be repeated until
182 wake time. Participants rated subjective sleep fragmentation using visual analogue scales upon
183 entering the laboratory.

184 ***Blood analysis***

185 All blood samples were immediately transferred into tubes treated with ethylenediaminetetraacetic
186 acid (EDTA) prior to centrifugation (10 min, 4000 x g, 4°C) before the plasma supernatant was
187 aliquoted and stored at -80°C for subsequent metabolite analysis. In the control trial the buffy coat
188 layer of the centrifuged bloods was removed and stored at -80°C for later genetic profiling. All plasma
189 samples were later analysed for plasma glucose, using a spectrophotometric analyser (RX, Daytona,
190 Randox Laboratories Ltd., Crumlin, UK; Inter-assay CV: <2 %) and insulin via enzyme-linked

191 immunosorbent assay (ELISA; Crystal Chem, IL, USA; Inter-assay CV: $13 \pm 3\%$, Intra-assay CV: 6
192 $\pm 2\%$).

193 ***DNA extraction and analysis***

194 DNA was extracted from the buffy coat layer using QIAamp DNA Blood Mini Kit following
195 manufacturer's instructions (Qiagen, Germany) and frozen at -80°C until analysis. Extracted DNA
196 was then analysed for the rs762551 SNP using a 5'-nuclease allelic discrimination assay (Taqman
197 drug metabolism genotyping assay SNP ID rs762551, C__8881221_40 [C/A], gene CYP1A2;
198 ThermoFisher Scientific, US).

199 ***Statistical Analysis***

200 Sample size estimations were performed using G*Power software v3.1.9.4. Based on differences in
201 plasma insulin following sleep deprivation ($D=1.58$), and considering the multi-level (i.e. 3 condition)
202 design of the study a sample size of 30 was deemed adequate to provide a 95% chance of detecting
203 such an effect at $\alpha=0.05$.

204 All in text values are reported as means with normalised confidence intervals, unless otherwise stated.
205 Normality of data was assessed using the Shapiro-Wilk test, with a paired t test or Wilcoxon's test
206 employed to analyse parametric data and non-parametric data respectively. A mixed model ANOVA
207 (condition, time, and condition x time) was used to examine differences in blood glucose and insulin
208 data, with *post-hoc* Bonferroni corrections applied in GraphPad Prism (GraphPad Software Inc.,
209 California, USA). Statistical significance was accepted at $p < 0.05$. Error bars shown on figures are
210 also normalised confidence intervals (CI) corrected for between-participant variation, such that the
211 magnitude of these CI's therefore directly infers the contrast between paired means at each time-point
212 rather than variance of individual values around the mean [19]. Using this approach, error bars not
213 overlapping their respective comparison would typically be deemed significantly different according
214 to conventional null hypothesis testing (i.e. $p < 0.05$). Incremental area under the curve (iAUC –
215 Trapezoid method [20]) and Matsuda Insulin sensitivity index (Matsuda index; [21]) were calculated
216 from plasma glucose and insulin data using Microsoft excel (Version 16.04848.1000, Microsoft,
217 Redmond, WA, USA). Updated homeostatic model of insulin resistance (HOMA2-IR; [22]) was
218 calculated using publicly available online software (<https://www.dtu.ox.ac.uk/homacalculator/>).
219 Distribution of the rs762551 SNP was tested for fit against the global expected distribution using a
220 Pearson's χ^2 test with 1 degree of freedom. All values for genotype data were taken from the
221 Fragmented+Coffee condition, with ΔiAUC calculated as the difference between Fragmented+Coffee
222 and Fragmented. As data for genotype was not paired, differences between means was compared

223 using standard 95% confidence intervals. Effects of trial order were assessed using two-way ANOVA
224 testing for effects of Condition, Sequence, or Sequence x Condition interactions [23, 24].

225 **Results**

226 ***Glycaemia, Insulinaemia, and Insulin Sensitivity***

227 Mixed model ANOVA revealed main effects for glucose for condition ($p < 0.01$), time ($p < 0.01$),
228 and condition x time ($p < 0.01$). Plasma glucose concentrations did not differ between conditions at
229 baseline and remained similar prior to ingestion of the oral glucose load (i.e. following caffeine or
230 hot water ingestion; **Figure 1A**). After ingestion of the oral glucose load, plasma glucose
231 concentrations rose to a greater extent in the Fragmented+Coffee *versus* the Fragmented and Control
232 conditions (8.61 [CI: 8.25-8.96] mmol·L⁻¹ *versus* 7.92 [CI: 7.57-8.28] mmol·L⁻¹, and 7.57 [CI: 7.21-
233 7.92] mmol·L⁻¹ respectively; $p < 0.05$). Plasma glucose concentration remained higher in the
234 Fragmented+Coffee condition relative to Control and Fragmented conditions at 120-mins ($p < 0.05$).
235 As such, plasma glucose incremental area under the curve (iAUC) was higher in the
236 Fragmented+Coffee condition relative to the Fragmented ($p = 0.02$) but not Control ($p > 0.05$)
237 Conditions (196.6 [175.4-217.7] *versus* 130.3 [114.3-146.4] *versus* 153.1 [137.0-169.1] mmol·L⁻¹·120min,
238 respectively).

239 Plasma insulin displayed effects of time ($p < 0.01$), but not condition ($p = 0.06$) or condition x time
240 ($p = 0.053$). Baseline plasma insulin concentrations were similar between conditions pre-OGTT
241 (**Figure 1B**). Following ingestion of the glucose load, insulin rose to a greater extent in the
242 Fragmented+Coffee condition, relative to both Control and Fragmented conditions (272 [251-293]
243 pmol·L⁻¹ *vs* 227 [206-248] pmol·L⁻¹ and 223 [202-244] pmol·L⁻¹) at 30-minutes ($p < 0.05$) (**Figure**
244 **1B**). Plasma glucose incremental area under the curve (iAUC) was not different in the
245 Fragmented+Coffee condition relative to the Fragmented ($p = 0.06$) and Control ($p = 0.08$) Conditions
246 (10035 [8892-11178] *versus* 7837 [7260-8980] *versus* 8425 [7848-9001] pmol·L⁻¹·120min,
247 respectively. Time to peak insulin, HOMA2-IR and Matsuda insulin sensitivity index were not
248 different between conditions (**Table 2**).

249 ***Order effect***

250 Analysis of Sequence x Condition interactions revealed no effect of first trial on the observed effect
251 of Fragmented+Coffee ($p = 0.101$). However, an order effect in plasma glucose iAUC was observed
252 whereby values in participants first trial were higher than both the second and third trial (197.0 [173.4-
253 220.6] *versus* 147.5 [125.7-169.3] *versus* 132.2 [106.5-158.0] mmol·L⁻¹·120 min).

254 **Subjective Sleep Quality**

255 Time to sleep (Median [Inter Quartile Range]; 23:30 [23:00-00:00] h vs 23:30 [23:00-00:01] h, vs
256 23:45 [23:05-24:10] h) and wake time (07:00 [06:52-07:17] h vs 07:00 [07:00-07:16] h vs 07:00
257 [06:55-07:20] h) did not differ between Control, Fragmented and Fragmented+Coffee conditions,
258 respectively. Subjective ratings of sleep fragmentation (i.e. “How fragmented was your night’s
259 sleep?”) were greater in the Fragmented, and Fragmented+Coffee conditions, relative to the Control
260 condition (83 [78-87] versus 81 [77-85] versus 8 [3-11] mm/100, respectively).

261 **Genotyping**

262 Of the n=26 genotyped, 15 participants were homozygous for the A allele, with the remaining 11
263 carrying the C allele (n=2 CC; n=9 AC). The distribution of genotypes was therefore as expected
264 within the given population and therefore did not deviate from the Hardy – Weinberg equilibrium
265 (Pearson’s χ^2 test with 1 df, $P > 0.05$).

266 **Secondary Analysis: Glycaemia and insulinaemia by genotype.**

267 The glucose incremental area under the curve (iAUC - AA: 208.5 [95%CI: 126.2-290.8] versus
268 AC/CC: 188.7 [95%CI: 103.9-273.4] mmol·L⁻¹·120min; $p = 0.54$) and peak plasma glucose
269 concentrations (AA: 8.86 [95%CI: 7.94-9.78] versus AC/CC: 9.13 [95%CI: 8.33-9.93] mmol·L⁻¹; p
270 = 0.71) did not differ between “fast metaboliser” (AA) and “slow metaboliser” (AC/CC) genotype.
271 Furthermore, Δ iAUC for glucose between the sleep fragmentation and coffee trials was similar
272 between “fast” and “slow” genotypes respectively (81.8 [18.5-145.1] versus 55.7 [-8.1-119.4]
273 mmol·L⁻¹·120 min; $p = 0.26$).

274 Similarly, peak insulin concentration (AA: 309.9 [220.7-399.1] versus AC/CC: 345.5 [283.7-407.4]
275 pmol·L⁻¹; $p = 0.84$), insulin iAUC (AA: 10234.5 [6720.2-13748.8] versus AC/CC: 10842.9 [6558.2-
276 15127.7] pmol·L⁻¹·120min; $p = 0.80$), and Δ iAUC for insulin did not differ between “fast metaboliser”
277 or “slow metaboliser” genotype respectively (2680.3 [423.0-4937.6] versus 2047.8 [-3447.06-
278 7542.6] pmol·L⁻¹·120min; $p = 0.75$).

279 **Discussion**

280 The current study demonstrates that one night of hourly sleep fragmentation had no effect on next-
281 day insulin sensitivity or glucose tolerance, relative to a habitual night of sleep, in young, healthy
282 men and women. However, consumption of caffeinated coffee after sleep fragmentation increased
283 glucose iAUC by ~50%.

284 A recent survey found that ~40% of people in the UK drink caffeinated coffee upon waking, and
285 therefore the coffee condition in the current study provided an important ecological comparison to
286 the sleep-fragmentation alone condition [8]. Previous studies have demonstrated acute reductions in
287 glucose tolerance when caffeinated coffee is ingested prior to assessment of glucose metabolism [10].
288 In the present study, glucose iAUC was ~50% greater following caffeinated coffee relative to sleep
289 fragmentation alone; this agrees with the one other study to have investigated the combination of
290 caffeinated coffee and sleep disruption [16], albeit in the reverse order (i.e. caffeine before *versus*
291 after sleep) and in relation to overall sleep deprivation (i.e. lower total duration) rather than the
292 fragmentation reported here. Indeed, in that previous study there was no habitual sleep control
293 condition and the caffeinated coffee was ingested during the sleep deprivation period; the present
294 study therefore extends those findings by isolating the independent effects of sleep deprivation and
295 caffeinated coffee, with the latter consumed following, rather than prior to sleep disruption.

296 Whilst not investigated in the current study, there are several potential mechanisms following
297 caffeinated coffee ingestion that may explain the reduction in glucose tolerance. Primarily, within a
298 circulating range of 25-40 mmol·L⁻¹ caffeine acts as an antagonist for adenosine receptors,
299 particularly in skeletal muscle where caffeine may inhibit glucose uptake via A1 adenosine receptor
300 antagonism [25-27]. Additionally, caffeine ingestion is a stimulant for the release of adrenaline,
301 which suppresses the action of insulin through β -adrenergic receptor activation [28-30]. It is also
302 worth considering the lipolytic effects of caffeine ingestion on glucose uptake into skeletal muscle.
303 The dose of caffeine provided in the current study is likely sufficient to induce lipolysis *prior* to the
304 OGTT, subsequently impairing glucose uptake into the muscle [31, 32]. Finally, the role of cortisol
305 in disruption of glucose metabolism must also be considered. Specifically, cortisol is elevated
306 following both caffeine ingestion [33], and sleep disruption [3], which elevates postprandial glucose
307 responses [34].

308 The rate of caffeine metabolism is affected by the rs762551 single-nucleotide polymorphism in the
309 CYP1A2 gene, with individuals classed as either fast (AA) or slow (AC/CC) metabolisers [35].
310 Recent evidence suggests that postprandial glycaemic and insulinaemic responses to both chronic and
311 acute caffeine ingestion are modulated by this polymorphism [14]. Despite observing the expected
312 distribution of the rs762551 SNP, no difference in the insulinaemic or glycaemic response to the
313 glucose load was found between genotype following caffeinated-coffee ingestion. While this may
314 suggest that the modulating effect of the rs762551 SNP was not present, this analysis was included
315 on an exploratory basis and thus the study was not directly powered to detect differences between

316 “fast” and “slow” metabolisers. Future work should therefore investigate the acute effect of this SNP
317 on the response to coffee both after a night of disrupted *versus* habitual sleep in a larger sample.

318 The current study observed no difference in insulin sensitivity or glucose tolerance following sleep
319 fragmentation relative to a habitual night of sleep. This is interesting considering that previous work
320 has shown even a single night of sleep restriction is sufficient to induce reductions in both peripheral
321 and hepatic insulin sensitivity relative to a habitual night of sleep [2]. In similar fashion to Gonnissen
322 *et al* [6], the current study observed one time-point at which postprandial plasma insulin concentration
323 was lower following Fragmented *versus* Control, which in the former was speculated to be due to a
324 difference in night-time glycogen use from waking. However, this cannot be directly concluded from
325 one time point alone. Speculatively, lack of postprandial differences in the current and previous study
326 [6] may be explained by the total magnitude of sleep disruption achieved through hourly sleep
327 fragmentation. In the current study, wake time was ~70-80 minutes total. Comparatively, Donga *et al*
328 [2] employed a broken sleep protocol (i.e. sleep time 2300-0100 h and 0500-0730 h) in healthy
329 lean subjects, observing a ~22% increase in endogenous glucose production alongside a ~20%
330 decrease in the rate of glucose disposal, indicative of reduced hepatic and peripheral insulin
331 sensitivity, respectively. Similarly, *two* nights of sleep reduction (~50% of habitual sleep duration:
332 442 ± 78 min *versus* 235 ± 34 min) also reduced Matsuda insulin sensitivity index by ~19% relative
333 to habitual sleep [4]. The total duration of sleep loss accumulated over one or more nights may
334 therefore be proportionate to the effect on postprandial glycaemia. Speculatively, this could be
335 explained by lesser disruption of slow-wave sleep (SWS - *i.e.* stage III of non-rapid eye movement
336 sleep) with the current protocol compared to previous studies. As SWS is thought to be the most
337 important mediator of metabolic, hormonal, and neurophysiological changes during sleep, studies
338 observing greater reductions in insulin sensitivity are likely to have done so through employment of
339 sleep disruption protocols that provide a greater degree of disruption to SWS [2, 4, 36]. Conversely
340 in the current study, participants were aroused from sleep every hour, which based on the average
341 length of each stage of sleep (~5-15 mins) and one sleep cycle (~90-110 mins) would potentially not
342 provide as much SWS disruption as previous literature [37].

343 Whilst the dose of caffeine ingested in the current is above that typically consumed in one cup of
344 coffee on a daily basis (~100-200 mg) [38, 39], studies employing lower doses (~60 mg) have
345 observed disrupted glucose control [10]. Despite this, a strength of the current study is the perhaps
346 more ecologically valid model of consumption following one night of sleep curtailment relative to
347 previous research [16]. Furthermore, the effects shown in this study are present in a relatively large
348 sample size for an acute study of this nature. One potential limiting factor in the interpretation of the
349 present results is the apparent order effect whereby mean plasma iAUC was higher in participants

350 first trial compared to both the second and third trials (197 [173-221] *versus* 148 [126-169] *versus*
351 132 [106-158] mmol·L⁻¹, respectively). However, repeated measures ANOVA revealed no interaction
352 effect between which trial participants performed first and the effect of Coffee on postprandial
353 glycaemia relative to Fragmented and habitual sleep. Simply put, the higher responses in the
354 Fragmented+Coffee condition were not due to the participants who performed the
355 Fragmented+Coffee trial first. Furthermore, the addition of a 4th condition (i.e. habitual sleep+coffee)
356 to the study would have provided an interesting comparison to fully assess the independent effects of
357 caffeinated coffee relative to sleep disruption. The lack of assessment of hormones potentially linked
358 to the mechanism of disruption (e.g. cortisol) also limit further discussion surrounding the effects
359 reported. Finally, the current study also did not strictly control for the menstrual phase in female
360 participants and this may have influenced our primary outcomes [40].

361 In summary, no effect of hourly fragmented sleep (totalling <80 mins) was found on postprandial
362 glucose and insulin responses to breakfast the next morning. However, the common approach of
363 consuming a strong caffeinated coffee following disrupted sleep resulted in a reduction in ~~insulin~~
364 ~~sensitivity and~~ glucose tolerance. Following a night of disrupted sleep, individuals should balance the
365 potential stimulating benefits of caffeinated coffee consumption with the potential to increase
366 postprandial glucose excursions.

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370 assistance with data collection.

371 **Conflict of interest**

372 None of the authors have any conflicts of interest to declare.

373 **Author contributions**

374 HAS –Design, data collection, analysis, write up
375 AH – Data collection, analysis, write up
376 JT – Data collection, write up
377 JPW – Analysis, write up
378 PH – Data collection, write up
379 OP – Data collection, write up
380 YCC – Data collection, write up
381 JGT – Design, data collection, write up
382 JAB – Design, data collection, analysis, write up
383

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Table 1. Summary of Participant Characteristics.

	Male (<i>n</i> =16)	Female (<i>n</i> =13)	Combined (<i>n</i> =29)
Age (y)	22 ± 1	21 ± 1	21 ± 1
Height (m)	1.81 ± 0.07	1.69 ± 0.07	1.78 ± 0.09
Body mass (kg)	82.2 ± 10.5	66.3 ± 11.5	75.3 ± 13.47
Body Mass Index (kg·m⁻²)	25.0 ± 2.6	23.2 ± 3.8	24.2 ± 3.3
Waist circumference (cm)	84.0 ± 6.4	73.3 ± 8.2	80.4 ± 8.7
Hip circumference (cm)	100.7 ± 6.8	97.1 ± 12.9	99.1 ± 10.0
Waist:Height (cm)	0.46 ± 0.03	0.43 ± 0.05	0.45 ± 0.04

Values are Mean ± Standard Deviation.

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	Control	Fragmented	Fragmented+Coffee
Peak Glucose (mmol·L⁻¹)	8.20 [7.93-8.47]	8.23 [7.96-8.50]	8.96 [8.70-9.22]*
Peak Insulin (pmol·L⁻¹)	265.1 [247.3-283.0]	235.4 [217.6-253.3]	310.5 [283.5-337.4]*
Time to Peak Glucose (min)	33 [29-36]	28 [25-32]	30 [27-33]
Time to Peak Insulin (min)	36 [32-40]	35 [31-38]	38 [35-42]
ISI Matsuda (au)	15.4 [14.3-16.3]	15.3 [14.2-16.3]	13.9 [12.9-14.9]
HOMA2-IR (au)	0.40 [0.26-0.54]	0.55 [0.41-0.68]	0.43 [0.28-0.58]

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Figure 1. A) Time course plasma glucose and B) Time course plasma insulin response to an oral glucose load following a habitual night of sleep (Control), a night of fragmented sleep (Fragmented), or sleep fragmentation + caffeinated coffee (Fragmented + Coffee). $*p < 0.05$